Understanding the Biology of Antimicrobial Resistance

Summary

To make science-based decisions about priorities, activities, and resource allocation to address the issue of antimicrobial resistance, a working knowledge of the biology of resistance is necessary. This section will review the basics of antimicrobial mechanisms of action and the development and spread of microbial mechanisms to resist antibiotics. Antimicrobial susceptibility testing of microbial isolates is an important tool for clinical use and for monitoring the prevalence of antimicrobial resistance. The broth microdilution method and the disk diffusion method are described and discussed.

Antimicrobial Classification and Mechanisms of Action

To understand how antimicrobial resistance occurs at the cellular level, it helps to review the various types of antimicrobials and their mechanisms of action. Antibacterials can be classified by biochemical structure and by mechanism of action. The major biochemical classes are beta-lactams, macrolides, aminoglycosides, tetracyclines, glycopeptides, sulfonamides, and quinolones. The major mechanisms of action of systemic antimicrobials involve inhibition of the following processess which are essential for bacterial growth and/or division: cell wall synthesis, nucleic acid replication, protein synthesis, and folate metabolism (Neu et al., 1996).

Inhibition of Cell Wall Synthesis

An essential component of the bacterial cell wall is a specific mucopeptide called a peptidoglycan. Multiple enzymes are required for peptidoglycan synthesis and attachment to the cell wall. Enzymes involved in the final stage of cell wall synthesis are called transpeptidases. Beta-lactam antimicrobials (penicillins, cephalosporins, carbapenems, monobactams) bind to transpeptidases and inhibit peptidoglycan formation, thus interfering with cell wall synthesis. These transpeptidase enzymes and some other bacterial proteins to which penicillins bind, are collectively called penicillin-binding proteins (PBPs). The PBPs are different for Gram-positive and Gram-negative bacteria and in anaerobic species. Beta-lactams are only efficacious against actively dividing bacteria, since that is when a new cell wall is being created.

Vancomycin is an example of a glycopeptide antimicrobial which also interferes with cell wall synthesis. It interrupts cell wall synthesis by forming a complex with residues of peptidoglycan precursors. Vancomycin and other glycopeptides also inhibit biochemical reactions in the cell wall catalyzed by transpeptidases and D,D-carboxypeptidases. Vancomycin has a large and complex chemical sturcture, and therefore is unable to penetrate the outer membrane of Gram-negative organisms. Beta-lactams and vancomycin, whose active site is the cell wall, can act synergistically with an aminoglycoside antimicrobial against enterococci. The cell wall active agents puncture the cell wall, allowing the aminoglycoside to get through the cytoplasm to reach its active target site, the ribosome.

Inhibition of Nucleic Acid (DNA) Replication

DNA gyrase is an enzyme that controls the folding or supercoiling of the DNA during DNA replication. It is essential for preventing the DNA molecule from becoming entangled during replication of circular chromosomes in bacteria. The quinolone class of antimicrobials bind to the DNA molecule-gyrase complex, inhibiting its function and leading to bacterial cell death. The original quinolone was naladixic acid, which only acts on aerobic Gram-negative species. The newer fluoroquinolones, such as ciprofloxicin, norfloxacin, and ofloxacin, have a much broader spectrum of activity.

Inhibition of Protein Synthesis

By interfering with protein synthesis taking place in the ribosome, several classes of antimicrobials are able to stop cell division. Bacterial ribosomes contain two subunits, the 50S and 30S subunits. Certain antimicrobials bind to one or both subunits, and cause misreading of the genetic code or formation of abnormal, nonfunctional protein complexes. Aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin) act primarily by binding to the 30S subunit. Tetracylines are another biochemical class of antibiotic which also bind to the 30S ribosome. Tetracylines are bacteriostatic rather than bactericidal, because their binding to the ribosome is transient. Several classes of antimicrobials inhibit the 50S ribosomal subunit. Macrolides (erythromycin), chloramphenicol and clindamycin are primarily bacteriostatic and attach reversibly to the 50S subunit and interfere with the linking of amino acids.

Inhibition of Folate Metabolism

Bacteria usually lack the ability to take up folic acid from the environment and must synthesize it internally. Trimethoprim and the sulfonamides interfere with folate metabolism by competitively blocking the synthesis of tetrahydrofolate. Trimethoprim and sulfonamides are usually administered together because trimethoprim potentiates sulfonamides.

Development and Spread of Mechanisms of Antimicrobial Resistance

There are two main aspects to the biology of antimicrobial resistance. One is concerned with the development, acquisition and spread of the resistance gene or factor itself. The other is the specific biochemical mechanism conveyed by this resistance gene or factor which thwarts the antimicrobial attack.

Resistance can be an intrinsic property of the bacteria itself which is possessed by all members of the genus, and renders it unaffected by a specific mechanism of an antimicrobial. Resistance can also develop as the result of a single or multiple step mutation, for example, which changes a ribosomal protein that was a target of an aminoglycoside antimicrobial. More commonly, resistance is not due to a chromosomal change event, but to the presence of extrachromosomal DNA which was acquired from another bacteria. This type of resistance is called plasmid-mediated (Neu et al., 1996). Bacteria can transfer chromosomal or plasmid DNA-containing resistance genes to another bacteria by conjugation, transduction, and transformation.

A plasmid is a circular body of doublestranded DNA which is separate from the chromosome and carries genes that encode various traits such as virulence and antimicrobial resistance (Fraimow et al., 1995). There are two types of plasmids based on their ability to transfer from one bacterium to another. Conjugative plasmids can transfer to other bacteria via sex pili, and nonconjugative plasmids cannot. Cell-to-cell contact is necessary for conjugation to occur and both donor and recipient end up with a copy of the plasmid. R-factors are plasmids that have traits for both conjugation and antimicrobial resistance (McManus, 1997). The transfer of plasmids by conjugation is an extremely important mechanism because transfer can occur in a broad range of bacterial species and can extend to highly unrelated organisms. A single plasmid can contain genes conferring resistance to multiple classes of antimicrobials.

Transduction occurs when chromosomal or plasmid DNA is transferred from one bacterium to another by bacteriophages (McManus, 1997). Bacteriophages are viruses that attack bacteria. Since bacteriophages have a very narrow host range, this is a less important method of resistance gene transfer.

Bacteria can pick up free or "naked" DNA from their environment by a process called transformation. The presence of free DNA is common after cell lysis, but the range of compatibility between the free DNA and the intact recipient bacteria is narrow (McManus, 1997). Therefore, transformation is not an important method of resistance gene transfer.

A transposon is a gene which contains an insertion sequence at each end. The insertion sequences allow the gene to jump to different locations on chromosomal DNA, from plasmid to plasmid or from chromosome to plasmid (McManus, 1997). The movement of a transposon is called transposition. Transposons are important because they can move resistance genes from a nonconjugative plasmid or chromosome to a conjugative plasmid, which can then be easily transferred to other bacteria. Another genetic element, called an integron, may be located on a plasmid or transposon. An integron contains one or more resistance genes (called gene cassettes) between two conserved DNA regions.

Biochemical Mechanisms of Bacterial Antimicrobial Resistance

There are four basic biochemical mechanisms by which bacteria resist the killing effects of antimicrobials: 1) alteration of the antimicrobial's target receptor molecule in the bacteria, 2) decreasing the assessibility of the antimicrobial to the target by altering entry of the antimicrobial into the cell or increasing removal of the antimicrobial from the cell, 3) destruction or inactivation of the antimicrobial, and 4) synthesis by the bacteria of a new metabolic pathway that is not inhibited by the antimicrobial (Neu et al., 1996). Resistance in bacteria arises through a multistep process, from low level to high level, unless a plasmid is acquired which already contains genes for full blown resistance (Levy, 1998). Multiple mechanisms of resistance can occur in a single isolate, leading to higher levels of resistance (Hawkey, 1998). Mechanisms of resistance are often specific to a particular antimicrobial agent in relation to a specific bacterial species, as the examples below will illustrate, and should not be generalized. A specific resistance mechanism operating in a specific bacterial species may also be a geographically local phenomenon.

Alteration of the Target Receptor

By altering the target receptor molecule, the antimicrobial is unable to bind and therefore does not have any effect. Altered penicillin-binding proteins (PBPs) are the cause of resistance in certain *Streptococcus pneumoniae* strains to penicillin G and also explain resistance of certain strains of *Staphylococcus aureus* to beta-lactamase stable penicillins. Resistance to fluoroquinolones is frequently associated with mutations in DNA gyrase, the target molecule, and therefore inhibit binding (Jenkins, 1996). Macrolide-lincomycin resistance in clinical isolates of staphylococci and streptococci is due to a biochemical change (methylation) in the 50S ribosomal subunit RNA, which decreases binding. The gene which causes this change in the ribosomal RNA is plasmid mediated and encoded on transposons.

Decreased accessibility of the antimicrobial agent to the target site can be accomplished in a number of ways. Membrane characteristics can inhibit the antimicrobial from crossing the membrane and entering the cell (decreased uptake), or the antimicrobial can be altered in its passage across the membrane so it can't bind its target. Resistant bacteria can also actively remove the antimicrobial from the cell (increased efflux). Tetracyline resistance is due to a decrease in the levels of drug accumulation caused by decreased uptake and increased efflux. This resistance is usually plasmid-mediated. Plasmids containing tetracycline resistance move among members of the Enterobacteriaceae, and also have moved between *S. Aureus, S. epidermidis, S. pyogenes, S. pneumoniae* and *S. faecalis*. Aminoglycoside resistance is largely due to the alteration of the compound in the periplasmic space by bacterial enzymes that acetylate, phosphorylate or adenylate aminoglycosides. This alteration of the compound leads to binding to the bacterial ribosomes and poor uptake into the cell. The genes coding for aminoglycoside altering enzymes are often found on transposons and have been identified in members of the Enterobacteriaceae and *P. aeruginosa, S. pneumoniae* and Gram-positive species such as *S. aureus, S. faecalis,* and *S. pyogenes* (Neu et al., 1996).

Destruction or Inactivation of the Antimicrobial

This resistance mechanism usually involves the hyperproduction of an enzyme that inactivates the drug. The most well-known example are the beta-lactamases, which are found in both Gram-negative and Gram-positive species. The clinically relevant Gram-positive bacteria that produce beta-lactamases are staphylococci and enterococci. Beta-lactamase resistance genes can be either chromosomally or plasmid-mediated and are widely distributed in nature. Chloramphenicol resistance is due to the presence of an intracellular enzyme called chloramphenicol transacetylate. This enzyme acetylates hydroxyl groups on the chloramphenicol structure which causes decreased binding to the 50S ribosome.

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Synthesis of a New Metabolic Pathway

Bacteria can produce a new enzyme that is not inhibited by the antimicrobial. Trimethoprim-sulfamethoxazole resistance is due to bacteria that produce a new dihydrofolate reductase not inhibited by trimethoprim and a new dihydropteroate synthetase not susceptible to sulfonamides.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing has two purposes. First, susceptibility testing is utilized clinically to predict the likely outcome of treating a patient's infection with a particular antimicrobial agent. Second, it can provide a quantitative measurement of susceptibility which can be used to monitor the emergence and prevalence of antimicrobial resistance. Currently, the two most popular susceptibility testing methods are the broth microdilution test and the disk diffusion test.

Broth Microdilution Test

The broth microdilution method is really the miniaturization and mechanization of one of the earliest methods of antimicrobial susceptibility testing, the tube-dilution method. Two-fold dilutions of antibiotics (e.g., 1 microgram/mL, 2 microgram/mL, 4 microgram/mL etc.) are prepared and added to individual wells in disposable plastic microdilution trays containing a liquid bacterial growth medium. The wells are then inoculated with a bacterial suspension of a standardized cell density. Following incubation for 16 to 20 hours, the trays are examined for evidence of bacterial growth in the form of turbidity. The lowest concentration of antimicrobial which prevents visible growth represents the MIC, or minimum inhibitory concentration.

These trays usually contain 96 wells, which allows 12 antibiotics to be tested in a range of eight two-fold dilutions in a single tray. Usually, pre-prepared microdilution antimicrobial trays or "panels", are purchased by most clinical microbiology laboratories. Buying pre-prepared panels saves on labor, time and reagent costs, but a disadvantage is the inflexibility of the antimicrobial selections available in the commercially prepared panels. It is also possible to automate the reading of the trays using photometer/tray readers.

The results are interpreted using the "interpretive criteria" published by the National Committee on Clinical Laboratory Standards (NCCLS). The NCCLS MIC interpretive criteria are established by careful analyses of the pharmacokinetics of a particular drug, microbiological testing, and clinical study results obtained during the the FDA pre-approval phase of commercial antimicrobial development (Jorgenson et al., 1998). "Breakpoint" MICs are established for each antimicrobial and bacterial species combination to categorize an organism as susceptible, intermediate or resistant. The MICs used for veterinary isolates are usually based on human breakpoints of clinical significance, which may lead to difficulty in interpretation of results. In general, although other factors must also be considered in the decision process, it is best to treat an infection due to a specific isolate with one of the antimicrobials having the lowest MIC for that isolate.

Disk Diffusion Test

The disk diffusion test is also known as the Kirby-Bauer procedure. A standardized inoculum is applied onto the entire surface of an agar medium in a large Petri plate. Uniform paper discs, each impregnated with a different antibiotic, or the same antibiotic in varying concentrations, are placed on the surface of the agar. The plates are then incubated for 16 to 18 hours. The antibiotic agent diffuses from the paper disk into the agar, thereby preventing the growth of the organism in a zone around the disc. The width of the zone is measured in millimeters and gives an indication of the sensitivity of the organism to the agent or agents being tested (Frobisher et al., 1974).

The results are interpreted by comparing the zone diameter with the interpretive criteria published by NCCLS. The interpretive criteria for the disk diffusion test categorizes the result as suceptible, intermediate or resistant. Therefore, a qualitative result is usually determined instead of a quantitative MIC. It is possible, however, to calculate an approximate MIC because the zone diameter correlates inversely with the approximate MIC for that antibiotic. The approximate MIC can be calculated with a computer software system which compares the zone-diameter values with standard curves for a species and drug using a linear regression formula (Jorgensen et al., 1998).

Sources of Error

An important source of error in susceptibility testing is that for certain bacterial species, differing qualities of bacterial growth may be due to the growth medium. A resistant organism may be misclassified as susceptible simply because it does not grow well in a particular culture medium. Another source of error is the amount of the inoculum. The ideal inoculum is related to both bacterial species and the specific antimicrobial. In some cases a heavy inoculum is appropriate, a light inoculum is appropriate in others (Phillips, 1998). For example, light inocula are needed for susceptibility tests using sulphonamides and trimethoprim.

Advantages and Disadvantages of Both Methods

The advantages of the disk diffusion procedure are cost effectiveness and flexibility. It is the least expensive method and is very flexible because the selection of the antibiotic discs is done by the user. The disadvantages include the lack of a quantitative result (MIC), and the lack of an automated procedure. Though the qualitative result (susceptible, intermediate, resistant) of this method is thought to be easily interpreted by clinicians, a quantitative result is becoming increasingly important in order to monitor small shifts in susceptibilty at the population level. Resistance often develops in degrees, for example, as additional resistance genes or factors are acquired by a specific strain. The MIC is a more precise measurement which can better reflect subtle changes in susceptibility, compared to the categorical result reported for the disk diffusion method (Phillips, 1998). Therefore, the current trend is toward the use of the broth microdilution method, especially the automated instrument methods (Jorgensen et al., 1998).

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